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Clinical Evaluation of Kethoxal Against Cutaneous Herpes Simplex

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A 2.5% preparation of kethoxal in cream was compared with the cream placebo for efficacy against cutaneous herpes simplex in a double-blind clinical study. The kethoxal formulation was not significantly more effective than the placebo. This conclusion was based on subjective impressions of the patients, observations by the physicians, and quantitative measurement of herpesvirus recovered from the lesions. It was suggested that the lack of clinical activity, in contrast to the marked activity against experimental infections in laboratory animals, resulted from the fact that high levels of herpesvirus were already present in the skin before symptoms were noted.

Kethoxal demonstrated outstanding activity when applied topically to cutaneous herpes simplex lesions on experimental animals (4). The compound, 3-ethoxy-2-oxobutyraldehyde hydrate, seemed a promising candidate for evaluation against "cold sores" in man. Animal toxicology studies indicated that it was safe to proceed with human topical tolerance testing. Phase 1 evaluation in prison volunteers demonstrated that a 2.5% concentration of the formulated drug was well tolerated topically. Studies examining efficacy against cutaneous herpes in man were then initiated in cooperation with four investigators. This report describes the clinical procedures and summarizes the results obtained in the efficacy study. Clinical results are further discussed in relation to controlled studies with an experimental cutaneous herpes infection in hairless mice and response of this laboratory infection to kethoxal treatment.

MATERIALS AND METHODS

General clinical procedure. Only adults with a history of recurrent cutaneous herpes simplex were accepted for the study. Each volunteer was given a coded tube containing drug or placebo, a list of instructions, and a form to be completed at the time of his next herpes episode. At the first symptoms of an incipient cold sore, the patient started treatment. He also visited the physician on day 1 for physical examination, attempted virus isolation, and blood sampling. The patient visited the physician again on days 2 and 3 for physical examination and attempted virus isolation. A post-treatment blood sample was obtained on day 3. The patient visited the physician for his final checkup 1 week after the first symptoms were noted.

Treatment procedure. On the form provided, the patient recorded the time when he first noted symptoms of a new cold sore. Symptoms were described by checking appropriate boxes on the form, i.e., tingling, itching, burning, pain, redness, swelling, blisters, oozing, soft scab, and dry crust. Topical treatment was started immediately, with the contents of the coded tube which contained either 2.5% kethoxal in cream or the cream alone (placebo). Self-treatment was administered every 2 hr up to a maximum of five treatments on day 1 and an additional four times at 3- to 4-hr intervals on day 2, at which time treatment was terminated.

Virus isolations. The physician examined the lesion at the time of each patient visit and recorded his findings on the appropriate form supplied to him. He also took samples for attempted virus isolation on days 1, 2, and 3 by gently swabbing the lesion with a sterile swab moistened with cell culture medium. The swab was broken off into a small serum bottle containing medium and stored immediately at −20 C. Muller's samples were assayed by E. C. Herrmann of the Mayo Clinic. All other virus isolates were sent to The Upjohn Co. and were assayed by the procedure described below.

Each sample was tested for herpes simplex virus (HSV) by inoculating 0.2 ml into each of two rabbit kidney tubes. One blind passage was made 5 to 6 days postinoculation from tubes showing no cytopathology. Fluids were harvested from positive tubes after disrupting the cells by freezing, and a sample was mixed with an equal volume of HSV immune serum prepared in rabbits. The original lesion swab samples from which confirmed HSV was isolated were then titrated by plaque assay on tissue culture dishes containing a monolayer of primary rabbit kidney cells. Two dishes were used for each 10-fold dilution and titers were calculated as plaque-forming units (PFU) per milliliter of swab sample.

TABLE 1. *Patient profile*

Patient data	Kethoxal	Placebo
Number	58	65
Age (mean year)	37	38
Sex (females)	30 (52)[a]	34 (52)
Race (white)	58 (100)	65 (100)

[a] Values in parentheses are percentages.

Investigators. The cooperating physicians and the number of usable case reports submitted by each were: R. D. Carr, Ohio State University, Columbus, 8 cases; S. A. Muller, Mayo Clinic, Rochester, Minn., 18 cases; W. A. Rye, The Upjohn Co., Kalamazoo, Mich., 85 cases; R. H. Grekin and P. W. Wang, Kalamazoo, Mich., 62 cases.

Blood studies. Hematocrit, hemoglobin, total white count, differential, serum glutamic oxalacetic transaminase, bilirubin, alkaline phosphatase, and creatinine were determined. Values obtained were compared to normal ranges for the respective testing laboratory.

Statistical evaluation of efficacy. The patient recorded the condition of the lesion at the time treatment was started, and the physician rated the lesion on the same scale on days 1, 2, and 3. This description was coded on a six-point scale showing the stage of development as: (i) no signs or symptoms; (ii) tingling, itching, burning, or pain, or all four; (iii) redness or swelling, or both; (iv) blisters; (v) oozing or soft scab, or both; (vi) dry crust. The progress of the lesion was assessed on days 1, 2, and 3 as "better," "same," or "worse," relative to its condition at start of treatment.

Hairless mouse studies. Mice were infected cutaneously with HSV and treated topically with kethoxal, and lesions were scored as previously described (4). In addition, virus yields from the mice were determined by immersing a sterile cotton swab into a 3-ml vial containing 1.5 ml of medium 199 with 5% heat-inactivated fetal calf serum and gently swabbing the affected area. The saturated swabs were broken at the stem, returned to the vials, sealed, and frozen at −55 C before titration in duplicate on primary rabbit kidney monolayers.

RESULTS

Of the 173 complete and usable case reports received from the four investigators, 123 described patients who started treatment within 3 hr of the time that symptoms of an incipient herpes lesion were first noted. Since early treatment should have the best chance of success, the statistical analyses were performed on these 123 case reports. There was no significant difference ($P > 0.05$) between the kethoxal- and placebo-treated groups with respect to age, sex, or race (Table 1). No blood changes due to kethoxal treatment were observed.

Efficacy was evaluated objectively by examining changes in appearance of the lesions as described above. There was no significant difference between the two treatments on any day (Table 2). Benefit of treatment was also assessed by considering the change in virus titer with respect to time. In making this evaluation, consideration was given to whether virus titer decreased or increased in successive samples and to the extent of this titer change. Only those cases were included in which more than one swab sample was taken and in which HSV was isolated from at least one of the samples. Assignment of such cases was made to one of the categories listed in Table 3, without knowing whether the patient had received drug or placebo treatment. Obviously, this procedure would not include patients in whom there may have been initial virus present which was eradicated by the treatment for the 3-day sampling period. Such sterilization may have occurred in some patients, but, if so, it was not a consistent response. This can be inferred from Table 3, which indicates that after treatment the virus titer increased in lesions of about half the virus-yielding patients, both in drug- and placebo-treated groups. It is apparent from the data in Table 3 that response of the two treatment groups was not significantly different based on changes in virus titers.

The two groups were compared each day by the chi square test for the number of subjects who had herpes present. All were included who had at least one sample taken for virus isolation. The totals vary from day to day because not all patients were sampled each day. The treatment groups were not significantly different (Table 4).

Each physician was also asked to record on the

TABLE 2. *Progress of lesion compared to its condition at start of treatment*

Treatment	Better on day			Same on day			Worse on day		
	1	2	3	1	2	3	1	2	3
Kethoxal	2 (5)[a]	3 (6)	8 (14)	33 (87)	27 (57)	10 (18)	3 (8)	17 (36)	39 (68)
Placebo	3 (6)	2 (9)	10 (16)	40 (78)	19 (41)	11 (17)	8 (16)	23 (50)	43 (67)

[a] Values in parentheses expressed as percentages.

TABLE 3. *Evaluation of treatment based on change in virus titers*

Treatment	Very beneficial	Beneficial	No effect	Became worse	Much worse
Kethoxal....	2 (7)[a]	11 (39)	3 (11)	7 (25)	5 (18)
Placebo.....	1 (3)	8 (25)	6 (19)	11 (34)	6 (19)

[a] Values in parentheses are expressed as percentages.

TABLE 4. *Isolation of herpesvirus*

Treatment	Herpesvirus present on day		
	1	2	3
Kethoxal....	15 (36)[a]	20 (46)	14 (29)
Placebo.....	17 (35)	23 (55)	17 (30)

[a] Values in parentheses are expressed as percentages.

TABLE 5. *Subjective evaluation of treatment*

Treatment	Benefit obtained				
	Very much	Much	Some	Little	None
Kethoxal....	8 (14)[a]	30 (34)	14 (24)	5 (9)	11 (19)
Placebo.....	11 (17)	25 (38)	12 (20)	3 (5)	13 (20)

[a] Values in parentheses are expressed as percentages.

case report form his estimate of the amount of benefit derived from the treatment. This subjective measure of efficacy showed no significant difference ($P > 0.05$) between the two groups (Table 5). Response to this question was also examined by limiting the cases to those in which herpesvirus was actually found (Table 6). Again, there was no significant difference between the two groups. Lesions at the treatment site developed in equivalent numbers of patients in the two groups (Table 7), and the examining physicians characterized all of these as "typical" herpes simplex lesions.

Mouse studies. Several experiments were performed in hairless mice as an aid to understanding the clinical results. In a typical experiment, 20 mice were infected cutaneously with HSV; no lesions appeared until the 3rd day after virus inoculation. All animals had 2+ or greater lesion scores on the 4th day after infection, and all animals were dead 1 week postinfection.

Table 8 presents data illustrating the favorable effects of kethoxal application to cutaneously infected hairless mice at different times after HSV inoculation. Excellent protection resulted from a single topical treatment of the infected area with kethoxal at any time between 1 and 6 hr after infection. The most effective time of treatment appeared to be about 4 hr after virus inoculation, as evidenced by lesion development in only 20% of the mice and an average virus yield in those mice developing lesions of less than 1% of that recovered from the controls on the 3rd day postinfection. In addition to prevention of lesion formation in 80% of the mice treated at 4 hr postinfection, it was noted that the lesions completely healed in six of the eight mice which did develop lesions, and these six mice lived for 1 month, at which time the experiment was terminated. Similar recovery occurred with mice treated with kethoxal at 2 hr postinfection, with somewhat smaller percentages recovering in the 1- and 6-hr groups (Table 8).

To determine the length of time kethoxal remained active on hairless mouse skin, groups of 10 mice each were scratched as for virus inoculation and then treated topically with 5% kethoxal in water. Separate groups were again scratched and inoculated with HSV at 15-min intervals thereafter for 1 hr. The results (Table 9) show a rapid loss of activity within 60 min of kethoxal application.

DISCUSSION

This study, as did a previous one with a different compound (5), clearly illustrates how essential placebo-controlled, blind-label procedures

TABLE 6. *Amount of benefit in those cases in which herpesvirus was found*

Group	Treatment	Amount of benefit				
		Very much	Much	Some	Little	None
Lesions ≤ 3 hr old	Kethoxal	1 (3)[a]	12 (34)	8 (23)	5 (14)	9 (26)
	Placebo	2 (6)	13 (41)	8 (25)	1 (3)	8 (25)
All lesions	Kethoxal	1 (2)	15 (30)	13 (26)	8 (16)	13 (26)
	Placebo	3 (7)	14 (33)	12 (28)	2 (5)	12 (28)

[a] Values in parentheses are expressed as percentages.

TABLE 7. *Lesion formation at treated site*

Treatment	Yes	No	No respones
Kethoxal.....	56 (97)[a]	2 (3)	0 (0)
Placebo.....	58 (89)	5 (8)	2 (3)

[a] Values in parentheses are expressed as percentages.

are when clinically evaluating an agent against cutaneous HSV. Thus, 72% of the kethoxal-treated patients appeared to derive at least some benefit from the treatment (Table 5), but the comparable figure for the placebo group was 75%, indicating the lack of a drug-related effect. Results obtained in drug-treated and placebo-treated groups were also similar for other parameters studied, and there was no evidence that kethoxal beneficially influenced the course of the infection. Since the compound was outstandingly active against cutaneous HSV infections in the laboratory (4), an explanation was sought for this lack of clinical activity.

The protocol was designed to initiate treatment as early as possible, i.e., when the patient felt the first symptoms of an incipient herpes lesion. Most of the patients started treatment within 3 hr of the time that symptoms were first noted, but over 50% of these patients indicated that their lesions had already progressed to the "blister" or vesicle stage. It would seem, therefore, that in the human disease appreciable virus replication has occurred before any symptoms of an approaching cold sore are noted. The burning, itching, and tingling, which often represent first symptoms, may indeed result from early interaction between viral antigen and its antibody; such antibody is invariably present in the serum of individuals who suffer from recurrent herpes lesions.

We can now consider, for purposes of comparison, the results obtained with experimental cutaneous herpes in hairless mice. If we plot on a single graph levels of virus recoverable from the inoculated area, "lesion score," and interval during which kethoxal therapy is effective, we

TABLE 9. *Duration of anti-herpesvirus activity of 5% kethoxal in water on hairless mouse skin*

Time of treatment (min before infection)	No. of mice	No. with lesion scores $\geq$ 2 on day 6
15	10	1
30	10	3
45	10	7
60	10	9
Control	10	10

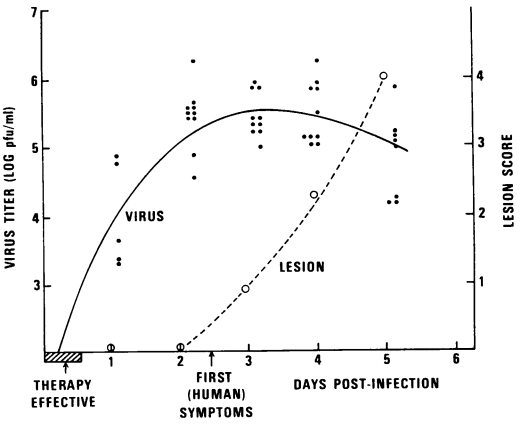

FIG. 1. *Cutaneous herpesvirus on hairless mice. Virus: each • represents the virus titer for an individual mouse. Titers less than 10^2 PFU/ml caused fewer than 10 points on days 1 and 5.*

TABLE 8. *Mice treated topically with a single application of 5% kethoxal in water at various times postinfection (PI) with herpesvirus (HSV)*

Treatment		No. of mice	No. with lesion scores $\geq$ 2 on day			Avg titer[a] on day 3 PI	No. of mice that recovered
Compound	Time (hr) PI		4	6	8		
Kethoxal	1	40	ND[b]	15	15 (38)[c]	3.7	9/15
	2	40	2	11	11 (28)	3.2	9/11
	4	40	2	8	8 (20)	2.8	6/8
	6	40	ND	19	19 (48)	ND	9/19
	8	40	ND	30	30 (75)	3.2	ND
	10	10	4	7	7 (70)	4.8	ND
Control		60	60	60	60 (100)	5.4	0/60

[a] Expressed as $\log_{10}$ PFU/0.5 ml of HSV in swab sample.
[b] Not determined.
[c] Values in parentheses are expressed as percentages.

find the interesting relationships shown in Fig. 1. These hairless mouse data suggest that it would be very unlikely that kethoxal treatment would be efficacious if it were delayed until first symptoms occur. In the hairless mouse, there is no sign of a lesion at 48 hr after virus inoculation, but most of the mice will have tiny vesicles at 72 hr post-infection, by which time the virus titer has reached its maximum. If the situation is comparable to man, there would probably be no symptoms of an impending lesion until at least 60 hr post-infection, i.e., shortly before the appearance of lesions. Kethoxal treatment is not effective in hairless mice, however, if delayed more than 10 to 12 hr after virus is inoculated.

It is not possible to establish direct correlations in a single host with respect to time of infection, appearance of first symptoms, and interval of effective therapy because, on the one hand, one cannot ethically infect volunteers with herpes virus and, on the other hand, one cannot question mice regarding their symptoms. The various human and mouse results taken together, however, indicate that virus titer in the cells is near maximum by the time symptoms are noted and that this is too late for treatment with kethoxal to have a beneficial effect on the course of the lesion. In fact, it appears unlikely that therapy with any antiviral agent would be effective in curing such a lesion; perhaps the most that could be expected is that a drug might prevent spread and development of new lesions. Of course, the great majority of recurrent herpes lesions are self-limiting and do not spread even without therapy.

Various agents have been claimed to be effective in treatment of cold sores, but none is yet accepted as possessing well established activity. The most thoroughly studied compound is 5-iodo-2′-deoxyuridine (IDU), but even here the clinical results are conflicting. MacCallum and Juel-Jensen reported activity with 5% IDU in dimethyl sulfoxide (DMSO) in a double-blind trial involving 16 patients (2). Average duration of the attack was reportedly reduced 64% in the IDU-treated group, but it was reduced 43% in those treated with DMSO only, indicating that the solvent alone was about two-thirds as effective as the drug-in-solvent in this rather small group of patients. Turnbull et al. also reported highly favorable results with topical IDU by using a 1% solution in DMSO (3), but these tests were uncontrolled and, therefore, suffer from the severe limitations imposed by the "placebo effect" noted earlier.

Kibrick and Katz, on the other hand, in a double-blind controlled study involving a total of more than 150 cases of herpetic lesions of the face, found no evidence that topically applied IDU was more beneficial than was placebo (1). In separate studies, they used 0.5% IDU in ointment and 0.1% IDU in 1.4% polyvinyl alcohol solution. IDU was ineffective in both studies, even when those cases in which treatment was initiated within 12 hr of onset were considered separately. Because of the disparaties in results obtained in these and other IDU studies, it is as yet impossible to arrive at any final conclusion concerning its efficacy against cutaneous HSV. Adequately controlled clinical data are not yet available for making valid judgments on other agents with claimed activity.

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